REMARKS

Claims 1-8, 10-14 and 16-35 are pending. Claim 9 has been cancelled. No new matter has been added.

Rejections under 35 U.S.C. §103

Claims 1-8, 10-14, and 16-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hyttinen *et al.*, in view of Rybak *et al.* According to the Examiner,

> Although Hyttinen et al. teaches general methods for making transgenic animals comprising enzyme fusion proteins and methods of making and isolating fusion proteins from the milk of transgenic mammals, Hyttinen et al. differs from the instant invention by not specifically teaching the production of a fusion protein comprising angiogenin. Rybak et al. supplements Hyttinen et al. by teaching nucleic acid expression constructs which encode a secretable fusion protein comprising a single chain antibody against the human transferring receptor and angiogenin (Rybak et al., abstract). Rybak et al. also teaches that the isolated fusion proteins are capable of inhibiting protein synthesis in human tumor cell lines (Rybak et al., page 3165). While Rybak et al. teaches the expression of the fusion protein in mammalian cell lines in vitro, the skilled artisan would have been motivated to express the fusion protein taught by Rybak et al. using a mammalian bioreactor system in order to produce larger quantities of the human fusion protein as taught by Hyttinen et al. Therefore, in view of the benefits of using a transgenic bioreactor to produce large quantities of a protein for use in humans, it would have been prima facie obvious to the skilled artisan to express the fusion protein taught by Rybak et al. using the transgenic bioreactors taught by Hyttinen. Further, based on successful use of transgenic bioreactors in expression large quantities of a variety of human proteins and enzyme containing fusion proteins as taught by Hyttinen et al., the skilled artisan would have had a reasonable expectation of success in expressing the fusion protein comprising the a single chain antibody against the transferrin receptor and angiogenin in the milk of a transgenic mammal according to the methods taught by Hyttinen et al.

The Examiner further states,

> The applicant argues that Hyttinen teaches a fusion protein comprising an inactive enzyme and therefore teaches away from the instant invention which recites an active enzyme. Hyttinen et al. however, clearly teaches that the fusion protein comprising the enzyme can contain an inactive enzyme or a biologically less active enzyme (Hyttinen et al., column 2, lines 32-54). Hyttinen therefore clearly teaches fusion protein which comprise an active enzyme.

Applicants respectfully traverse this rejection. The instant claims are directed to methods of making a biologically active fusion protein in the milk of a transgenic mammal. The fusion protein includes a first and a second member where the second member is an enzyme produced in its biologically active form and the fusion protein is expressed at levels of at least 0.1 mg/mL. In addition, the claims are directed to a transgenic mammal producing such fusion proteins. Claims 2, 3, 4, 5, 10, 13, 18, 19, 24, 25, 26, 27, 31, 32, and 33 further recite that the first member of the fusion protein is a targeting molecule such as an immunoglobulin, e.g., an immunoglobulin to a tumor antigen.

The Hyttinen *et al.* reference teaches the use of transgenic mammals to produce a less active or inactive protein as part of a fusion protein in milk. Specifically, Hyttinen et al. state that polypeptides (such as enzymes) are produced "as fusion proteins that are less active than said biologically active polypeptide in its free form, or non-active. The activity is diminished or removed by having the biologically active polypeptide produced as a fusion protein" (column 2, lines 41-44). Applicants have amended the claims to insert the term "biologically" active form. Although the claims previously recited this by referring to the enzyme being produced in its active form, Applicants added the term "biologically" to emphasize to the Examiner that nothing in Hyttinen et al. teaches or suggests that an enzyme produced as part of a fusion protein would be in its biologically active form. This is clear from the teachings of Hyttinen which explicitly state that the polypeptide should be <u>inactive</u> or have a <u>diminished biological activity</u> compared to the polypeptide in its free form. (emphasis added) Hyttinen et al. further state, at column 4, lines 52-55, that the fusion protein includes a "recombinant DNA encoding a fragment or intact milk or non-milk protein [to provide] the other part of the fusion protein. This polypeptide is used to <u>reduce the biological activity of the polypeptide</u>." (emphasis added) The focus in Hyttinen *et al.* is to use the transgenic bioreactor to express a high level of inactive or less active protein since "[s]evere side effects are ... probable when producing potent polypeptides like growth factors, cytokines or enzymes..." See column 2, lines 23-24. It is clear this is very different from the claimed invention which require that the second member- the enzyme portion- of the fusion protein be produced in its biologically active form. Thus, unlike Hyttinen, the claimed first member does not reduce the activity of the second member of the fusion protein. Thus the teachings of Hyttinen *et al.* differ substantially from the instant claims. There is nothing in the

Hyttinen *et al.* reference that would motivate a skilled artisan to produce fusion proteins in which the enzyme portion is in a biologically active state. In fact, it actually teaches that producing expression of a fully active enzyme portion is unfavorable.

The Rybak *et al.* reference discloses the production of a fusion protein consisting of an immunoglobulin heavy chain and angiogenin in a culture system. Rybak et al. disclose that this fusion protein is secreted into cell culture at very low levels, namely 1-5 ng/mL (0.001-0.005 mg/mL) based on the reactivity to anti-human IgG antibodies, and 1-2 ng/mL (0.001-0.002 mg/mL) based on angiogenin immunoreactivity. See column 5, lines 6-9. There is nothing in Rybak et al. which would teach or suggest that secretion of such a fusion protein could be improved. Thus, neither the Hyttinen *et al.* reference nor the Rybak *et al.* reference alone or in combination provide any indication of a likelihood of success in expressing reported poorly expressed protein in the milk of a transgenic mammal in which secretion is an essential step in recovering the fusion protein at levels as high as at least 0.1, 0.5 or 1 mg/mL (at least 100 times greater expression. In view of the very low expression levels of this fusion protein in cell culture (see, e.g., Rybak *et al.*), it would be unexpected that this fusion protein would be secreted at such high levels in the milk of transgenic mammals.

The Examiner asserts that the motivation to combine these references can be found in the discussion in Hyttinen et al. that "transgenic bioreactors...[express] large quantities of a protein for use in human." However, Hyttinen et al. provide no suggestion that a protein secreted at very low levels (i.e., less than 0.005 mg/mL) an cell culture would be produced in milk , a system requiring secretion to work at any significant level. The fact that Hyttinen et al. disclose using mammals "which produce large quantities of milk and have long lactation periods," provides absolutely no suggestion that the claimed fusion proteins, which were secreted so poorly in cell culture, could be secreted at levels of 0.1 mg/mL in milk. This was an unexpected result of the claimed invention.

Moreover, the Examiner's reliance on the Example provided in Hyttinen of an inactive EPO being produced as part of a fusion protein with β-lactoglobulin, a native milk protein, still would not suggest that expression of non-native milk proteins, such as immunoglobulin fused to a non-milk enzyme provided in the currently claimed invention, would be successfully expressed

at high levels. A native milk protein would be expected to be expressed at high levels in milk but there is no reason to expect that a protein not native to milk would be highly expressed in milk.

Thus, it is clear that neither the Hyttinen *et al.* reference nor the Rybak *et al.* reference, alone or in combination, teach or suggest the claimed invention. Moreover, there is nothing in either of these references that would motivate one skilled in the art to combine the teachings of these references to arrive at the claimed invention. Lastly, the expression levels of the fusion protein obtained in the milk of transgenic mammals, as presently claimed, would be unexpected in view of the very low expression levels of these proteins in other expression systems. Therefore, the Hyttinen *et al.* reference and the Rybak *et al.* reference do not render the claimed invention obvious. The Applicants respectfully request that the Examiner withdraw this rejection.

Applicant asks that all claims be allowed. Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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Laurie Lawrence

Laurie Butler Lawrence
Reg. No. 46,593

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

Version with markings to show changes made

In the claims:

Claim 9 has been cancelled.

Claims 1 and 16 have been amended as follows:

1. (Amended) A method of making a transgenic fusion protein which includes a first member and a second member, wherein the second member is an enzyme, the method comprising: providing a non-human transgenic mammal which includes a transgene which provides for the expression of the fusion protein in the milk of the mammal; and allowing the transgene to be expressed, thereby providing the fusion protein in the milk of the mammal wherein the second member is in biologically active form and the fusion protein is produced at levels of at least about 0.1 mg/ml in the milk of the mammal.

16. (Amended) A non-human transgenic mammal which includes a transgene that encodes a fusion protein, the transgene comprising: a mammary epithelial specific promoter, a nucleotide sequence which encodes a signal sequence which can direct the secretion of the fusion protein, and one or more nucleotide sequences encoding the fusion protein, wherein the fusion protein includes a first member and a second member, the second member is an enzyme produced in the milk of a transgenic mammal in biologically active form, and the fusion protein is produced in the milk of the transgenic mammal at a concentration of at least about 0.1 mg/ml.